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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)

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☐ *Additional Inventors are being named on the____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

Gene Encoding Resistance to Acetolactate Synthase-Inhibiting Herbicides

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☒ Customer Number 30743 →

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages*	15	☐ CD(s), Number	
☒ Drawing(s) *Number of Sheets*	3	☐ Other (specify)	
☐ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☒	Applicant claims small entity status. See 37 CFR 1.27.	FILING FEE AMOUNT ($)
☒	A check or money order is enclosed to cover the filing fees	
☒	The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number 50-2041	$80.00
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE

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Date 1-9-03

REGISTRATION NO. *(if appropriate)* 32,635

Docket Number: 01640393PR

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

P19SMALL/REV05

Gene Encoding Resistance to Acetolactate Synthase-Inhibiting Herbicides

ABSTRACT

The present invention describes a novel mutation in the acetolactate synthase (ALS) gene, which is the target site for several important classes of herbicides including the sulfonylureas, imidazolinones, pyrimidinyloxybenzoates, and triazolopyrimidines. This mutation results in a single amino acid change within a conserved region of the ALS amino acid sequence. This mutation creates an ALS enzyme with a unique pattern of cross-resistance to all sulfonylurea, imidazolinone, pyrimidinyloxybenzoate, and triazolopyrimidine herbicide chemistries.

DESCRIPTION OF THE INTELLECTUAL PROPERTY

4. If an INVENTION, provide a complete description and identify and describe the novel or unusual features.

Herbicides have simplified weed management in agriculture and provide a highly effective means of keeping weed populations at acceptable levels. However, crop sensitivity to numerous herbicides limits the use of these herbicides to tolerant crops only. Certain herbicides currently registered for use in crops still result in injury even at normal use rates. Crop injury increases when higher application rates are required to manage large weeds or heavy infestations that are beyond control with normal use rates. In extreme situations, the only effective herbicides available may result in significant crop injury. Furthermore, residual herbicides remaining in the soil are often a problem with rotation to a sensitive crop the following season, which may hinder the use of effective herbicides based on rotational restrictions. Modification of crop plants to create herbicide resistance has been an effective tool to increase weed control, minimize crop injury, allow applications of herbicides in crops with previous sensitivity, reduce herbicide inputs, and make use of more environmentally sound herbicide options. Transgenic crops resistant to a specific herbicide have been developed by transformation with target enzymes that are insensitive to that specific herbicide.

Acetolactate synthase (ALS) is an enzyme that catalyzes the initial step in the branched chain amino acid biosynthetic pathway. ALS is the target site of several classes of unrelated herbicide chemistries, including sulfonylureas (SU), imidazolinones (IMI), pyrimidynloxybenzoates (POB), and triazolopyrimidines (TP) (Table 1). Currently, ALS-inhibiting herbicides comprise the largest mode-of-action group in use due to broad-spectrum weed control in a variety of crops at very low application rates. In addition, ALS-inhibiting herbicides have very low mammalian toxicity. These characteristics have increased the importance of these herbicides in production agriculture and have attracted the development of ALS-resistant crops.

A single nucleotide mutation in the ALS enzyme is capable of conferring resistance to ALS-inhibiting herbicides. Mutations have been identified in five highly conserved domains along the DNA sequence coding for the ALS enzyme in higher plants. Each domain contains a single

variable residue, that when substituted, confers resistance to ALS-inhibiting herbicides. In most cases, a single substitution results in target-site cross-resistance differences between ALS-inhibiting herbicide chemistries (Table 2). A substitution reported at Ala_{133} in domain C of common cocklebur resulted in resistance to IMI herbicides only. The identical mutation was found in a commercial field corn hybrid, ICI 8532 IT, and sugar beet line Sur, which are crops resistant to only IMI herbicides (Bernasconi et al., J. Biol. Chem. (1995) 270:17381-17385; Wright et al., Weed Sci. (1998) 46:13-23). Substitutions at Pro_{197} in domain A have resulted in a high levels of resistance to SU herbicides with little or no resistance to IMI herbicides (Guttieri et al., Weed Sci. (1992) 40:670-676; Guttieri et al., Weed Sci (1995) 43:175-178; Boutsalis et al., Pestic. Sci. (1999) 55:507-516). A domain E mutation of Ser_{670} to Asp resulted in a high level of resistance to IMI herbicides with low SU resistance (Devine and Eberlein, Herbicide Activity: Toxicology, Biochemistry and Molecular Biology (1997) 159-185). High-level cross-resistance between ALS-herbicide chemistries has been shown previously with field isolated common cocklebur (*Xanthium strumarium*) biotypes exposed to several years of ALS selection pressure (Bernasconi et al., J. Biol. Chem. (1995) 270:17381-17). The isolated protein from one resistant biotype had a Trp_{552} to Leu mutation as compared to the susceptible population. This mutation corresponded to the Trp_{542} to Leu mutation in a commercial corn hybrid, Pioneer 3180 IR, which exhibited broad-range tolerance to ALS-inhibiting herbicides. A second cocklebur field isolate had a substitution of Ala_{183} to Val in Domain D that conferred similar cross-resistance patterns to the mutation found in domain B (Woodworth et al., Plant Physiol. (1996) 111:415).

Seeds from a smooth pigweed (*Amaranthus hybridus* L.) population (R11-AMACH) were collected from a field in southeastern Pennsylvania where extreme ALS-inhibitor herbicide selection pressure was imposed over a several year period within continuous soybean production. R11-AMACH was selected naturally with ALS-inhibiting herbicides representative of the SU, IMI, and TP herbicide chemistries.

To establish levels and patterns of ALS resistance, R11-AMACH and an ALS susceptible smooth pigweed biotype (S-AMACH) were screened in the greenhouse with various rates of the ALS-inhibiting herbicides, chlorimuron (SU), thifensulfuron (SU), imazethapyr (IMI), pyrithiobac (POB), and cloransulam-methyl (TP). Rates evaluated were based on a log10 scale that included 0, 1/100x, 1/10x, 1x, 10x, and 100x, where 1x corresponds to the normal use rate in the field. R11-AMACH responded differently to the rate increase as compared to S-AMACH. With all herbicides applied, R11-AMACH showed high-levels of resistance based on the response of the S-AMACH. Visual control, height, biomass, and biomass reduction are presented separately for chlorimuron (Table 3), thifensulfuron (Table 4), imazethapyr (Table 5), pyrithiobac (Table 6), and cloransulam (Table 7). Evaluations and measurements were recorded 3 weeks after herbicide treatment (WAT). Visual control was based on a scale of 0-99%, where 0% represents no control and 99% represents complete control. Biomass represents plant dry weights recorded several days after plants were harvested. Biomass reduction was calculated based on the amount of biomass reduced by herbicide treatment compared to the untreated plant biomass. Results show R11-AMACH resistance levels above 100 times the normal use rate to both SU herbicides, chlorimuron and thifensulfuron, and to the TP herbicide, cloransulam-methyl. Resistance levels to IMI and POB herbicides, imazethapyr and pyrithiobac, respectively, were greater than 10 times the normal use rate. Illustrations of the R11-AMACH biotype response 3 WAT are presented for the various rates of chlorimuron (Figure 2), thifensulfuron

(Figure 3), imazethapyr (Figure 4), pyrithiobac (Figure 5), and cloransulam (Figure 6). Results indicated R11-AMACH has target-site cross-resistance to four classes of structurally unrelated chemistries of ALS-inhibiting herbicides, namely SU, IMI, POB, and TP.

To establish why R11-AMACH exhibited high-levels of resistance to four classes of ALS-inhibiting herbicides, ALS enzymes from R11-AMACH and S-AMACH were isolated and sequenced. The R11-AMACH nucleotide sequence is presented in Figure 6a and the corresponding protein in Figure 6b. The nucleotide sequence of S-AMACH is presented in Figure 7a and corresponding protein in Figure 7b. No nucleotide differences were observed between R11-AMACH and S-AMACH in any of the five previously reported conserved domains known to confer ALS resistance in higher plants. However, a single amino acid difference was discovered in the R11-AMACH biotype ALS that occurred in a conserved region previously unreported to confer ALS resistance in higher plants (Figure 8). This region consists of the amino acid residues, GVRFDDRVTGK, which are identical to that of corn (*Zea mays*), cotton (*Gossypium hirsutum*), canola (*Brassica napus*), rice (*Oryza sativa*), tobacco (*Nicotiana tabacum*), and *Arabidopsis thaliana*. The conserved region corresponds to positions 379 to 389 of the *Arabidopsis* ALS coding sequence. At position 375 of the smooth pigweed ALS amino acid sequence, S-AMACH contained an aspartic acid residue, whereas R11-AMACH contained a glutamic acid residue (Figure 8). The amino acid change was a result of a single point mutation in the nucleotide sequence of R11-AMACH where A replaced G in the sequence GAG encoding for aspartic acid (underlined residue is point of mutation). This invention provides a functional ALS enzyme in higher plants with the amino acid sequence described in Figure 6b, which confers resistance to ALS-inhibiting herbicides comprising four structurally unrelated chemistries.

5. What is the existing technology/art to which you are comparing?

The existing technology encompasses functional enzymes in higher plants with ALS-inhibiting herbicide resistance characteristics. As stated previously, the majority of mutations in the ALS gene that confer resistance to ALS herbicides are specific for a certain ALS-chemistry, conferring high levels of resistance to one or two of the ALS-chemistries. Two ALS gene mutations in previously reported domains confer high levels of cross-resistance to four ALS-inhibiting herbicide chemistries, specifically SU, IMI, POB, and TP (Bernasconi et al., J. Biol. Chem. (1995) 270:17381-17385; Bernasconi et al., (1995) U.S. Patent No. 5,633,437; Woodworth et al., Plant Physiol. (1996) 111:415). Both mutations were reported to confer cross-resistance in common cocklebur and one to a commercially available corn hydrid, Pioneer 3180R. Other crops have been transformed with known ALS mutations that confer resistance specifically to SU or IMI herbicides. Transformed crops with a sulfonylurea resistant ALS enzyme include cotton, soybean, corn, sugarbeet, flax, tobacco, and canola. Imidazolinone resistant ALS enzymes have been transformed into corn, wheat, and rice. Site-directed mutagenesis of the yeast ALS gene at position 384 where valine, asparagine, or glutamic acid were introduced has shown to result in a SU resistant transformant (Bedbrook et al., (1995) U.S. Patent No. 5,378,824). Position 384 of yeast corresponds to position 375 of the smooth pigweed ALS amino acid sequence. Introduction and expression of a glutamic acid mutation at position 384 has not been demonstrated in higher plants under laboratory conditions.

6. How does your invention differ from present technology, what problems does it solve, or what advantages does it posses? (This should be written so someone skilled in the art can understand it.)

The present invention provides a functional ALS enzyme resistant to four structurally unrelated ALS-inhibiting herbicide chemistries. ALS resistance is conferred by a single amino acid mutation in a conserved region previously unreported along the ALS gene in higher plants. Two separate mutations in other conserved regions of the ALS gene have been reported to confer cross-resistance to four classes of ALS-inhibiting herbicides. The ALS-resistant enzyme disclosed provides another option to confer broad-based ALS-resistance to crop plants. Cross-resistance characteristics conferred to crop plants would allow the option to apply any ALS-inhibiting herbicide, which would increase the number of herbicides available to apply on that crop for the given weed spectrum. Crop plants with single ALS-resistance characteristics are advantageous, but herbicide options are specific for that chemistry of ALS-inhibiting herbicides or other herbicides to which the crop has natural tolerance. Currently, many crops are tolerant to a limited number of herbicides, which usually results in a high cost of weed control.

7. If not indicated previously, what are the possible uses and markets of the INTELLECTUAL PROPERTY? In addition to immediate applications, are there other uses that might be realized in the future?

ALS-inhibiting herbicides comprise the largest mode-of-action group on the market today and include four chemically unrelated herbicide families that provide the capability for a broad-range of weed control selectivity. The present invention can be transformed into crop plants to confer selective resistance to four classes of structurally unrelated chemistries of ALS-inhibiting herbicides. A crop transformed to tolerate all four classes of ALS-inhibiting herbicides would provide an option to apply any of the numerous ALS herbicides, which would broaden the spectrum of weeds controlled. This invention has a potential to be utilized in any crop species, but would specifically be advantageous to crops with few herbicide options or sensitivity to residual carry-over from the previous season. Further transformations may be possible to combine the disclosed invention with another herbicide resistance trait to confer resistance to ALS-inhibiting herbicides, as well as other herbicide modes of action. This "stacking" of herbicide resistance traits will further broaden herbicide options for the given weed population density and spectrum. ALS-inhibiting herbicides are currently a major portion of the herbicide market; therefore, many major chemical companies have focused research on these compounds and generation of ALS-resistance crops. New ALS-inhibiting herbicide chemistries may be developed in the future to which this invention provides a mechanism of resistance, thereby further increasing the value of this invention.

2. Has the INVENTION been tested experimentally? _x_Yes ___No
Are experimental data available? _x_Yes ___No
If possible, attach a copy of the key experimental results. If necessary, who should be contacted for access to the data?

Key experimental results are attached as Tables 3, 4, 5, 6, and 7 and Figures 1, 2, 3, 4, 5, 6a, 6b, 7a, 7b, and 8.

3. Are there known INVENTIONS by others that are related to this one? Please describe, including literature references to relevant patents and publications that most closely describe the state of the related art prior to your invention.

Site-directed mutagenesis of the yeast ALS gene with valine, asparagine, or glutamic acid at the same position as the current invention resulted in a SU resistant transformant (Bedbrook et al., (1995) U.S. Patent No. 5,378,824). In this case, patterns of cross-resistance to ALS chemistries were not evaluated. Furthermore, introduction and expression of glutamic acid at this position has not been demonstrated in higher plants under laboratory conditions. Two ALS enzymes isolated from a higher plant conferred cross-resistance to four-chemistries of ALS-inhibiting herbicides. The mutations conferring resistance occurred in a different region than the current invention (Bernasconi et al., J. Biol. Chem. (1995) 270:17381-17385; Bernasconi et al., (1995) U.S. Patent No. 5,633,437; Woodworth et al., Plant Physiol. (1996) 111:415). Both mutations were reported to occur naturally in common cocklebur and one was reported to confer resistance in a commercially available corn hybrid, Pioneer 3180R.

1. Please describe briefly the impact this INTELLECTUAL PROPERTY is likely to have on the field of endeavor (i.e., marginal improvement, significant change, revolutionary upheaval, creates new field, etc.) and why.

The intellectual property disclosed will improve upon the current ALS-resistant enzymes that confer resistance to only a single chemistry of ALS-inhibiting herbicides in crops. The intellectual property will provide the opportunity to apply four ALS-inhibiting chemistries on a transformed crop. Greater flexibility in herbicide application will expand the ALS-inhibitor herbicide market, as well as provide more herbicide options for weed control while upholding crop safety.

2. Please describe briefly the stage of development of the INTELLECTUAL PROPERTY (i.e., conceptual idea, theoretical design, prototype, complete product/process, outline, rough draft, finished work of authorship, ready for commercial testing/marketing, etc.) and give an estimate of the nature and amount of work that still remains to be done before a commercial venture/product is obtained.

Cross-resistance to four structurally unrelated ALS-inhibiting herbicide chemistries has been exhibited on the whole-plant level with the R11-AMACH population. The ALS gene has been isolated from R11-AMACH and confirmed to contain a single amino acid difference from an ALS-susceptible smooth pigweed population. This single amino acid difference is responsible for the ALS resistance patterns observed. Further work is underway to produce a transgenic plant with this ALS-resistant enzyme.

Table 1. Representative examples of sulfonylurea, imidazolinone, pyrimidinyloxybenzoate, and triazolopyrimidine ALS-inhibiting herbicides and corresponding chemical names.

ALS-Inhibitor Family	Common Name	Chemical Name
Sulfonylurea	chlorimuron	2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino] sulfonyl]benzoic acid
	thifensulfuron	3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino] sulfonyl]-2-thiophenecarboxylic acid
	trifloxysulfuron	N-[(4,6-dimehoxy-2-pyrimidinyl)carbamoyl]-3-(2,2,2-trifluoroethoxy) -pyridin-2-sulfonamide
	nicosulfuron	2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] -N,N-dimethyl-3-pyridinecarboxamide
Imidazolinone	imazethapyr	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl] -5-ethyl-3-pyridinecarboxylic acid
	imazaquin	2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl] -3-quinolinecarboxylic acid
	imazapyr	(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl] -3-pyridinecarboxylic acid
Pyrimidinyloxybenzoate	pyrithiobac	2-chloro-6-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid
Triazolpyrimidine	cloransulam	3-chloro-2-[[(5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-c]pyrimidin-2yl)sulfonyl]amino]benzoic acid
	flumetsulam	N-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide

Table 2. Common ALS mutations and corresponding levels of resistance conferred to SU, IMI, and TP herbicides (Devine and Shukla, Crop Prot. (2000) 19:881-889).

Mutation	Domain	Domain Sequence	Resistance Level			Reference
			SU	IMI	TP	
Ala_{122} to Thr	C	VFAYPGGASMEIHQALTRS	Low zero	High	Low zero	Bernasconi et al. (1995)
Pro_{197} to Ala	A	AITGQVPRRMIGT	High	Zero	Mod low	Boutsalis et al. (1999)
Pro_{197} to Thr			High	Low zero	-	Guttieri et al. (1995)
Pro_{197} to His			High	Mod	Low	Guttieri et al. (1992)
Pro_{197} to Leu			High	Mod low	High	Guttieri et al. (1995)
Pro_{197} to Arg			High	-	-	Guttieri et al. (1995)
Pro_{197} to Ile			High	Mod low	Mod low	Boutsalis et al. (1999)
Pro_{197} to Gln			High	-	-	Guttieri et al. (1995)
Pro_{197} to Ser			High	Zero	High	Guttieri et al. (1995)
Ala_{205} to Asp	D	AFQETP	High	-	-	Hartnett et al. (1990)
Trp_{591} to Leu	B	QWED	High	High	High	Boutsalis et al. (1999)
Ser_{670} to Asp	E	IPSGG	Low	High	Zero	Devine and Eberlein (1997)

Abstract 1. A mutation in the ALS gene confers resistance to four classes of ALS-inhibiting herbicides in smooth pigweed (*Amaranthus hybridus*). Cory M. Whaley, Dr. James H. Westwood, and Dr. Henry P. Wilson. Virginia Polytechnic Institute and State Univ., Blacksburg, VA 24061.

Smooth pigweed seeds were collected in 1999 from a field in southeastern Pennsylvania where acetolactate synthase (ALS)-inhibiting herbicide selection pressure was imposed over several consecutive years within continuous soybean production. Herbicide applications each year consisted of ALS-inhibitors representative of the imidazolinone, sulfonylurea, or triazolopyrimidine classes. Greenhouse studies were conducted to evaluate the response of the ALS-resistant (R) biotype and an ALS-susceptible (S) biotype to four ALS-inhibiting herbicide classes. Results indicated the R biotype was cross-resistant to representatives of the sulfonylurea (chlorimuron and thifensulfuron), imidazolinone (imazethapyr), pyrimidinyloxybenzoate (pyrithiobac), and triazolopyrimidine (cloransulam-methyl) classess. Comparisons of ALS gene sequences from R and S plants revealed no differences in the five ALS domains previously characterized as conferring resistance to ALS-inhibitor herbicides. However, a single amino acid mutation was found in the R biotype ALS gene in a conserved region previously unreported from herbicide resistant plants. The mutation in the R biotype was a single amino acid change in a region thought to be important in binding a cofactor, which may indirectly affect herbicide binding.

Table 3. R11-AMACH and S-AMACH visual control, height, biomass, and biomass reduction 3 WAT with various rates of chlorimuron (SU).

	Visual Control		Height		Biomass		Biomass Reduction	
RATE	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH
	%		cm		g		%	
0	0	0	33.8	30.5	3.80	3.43	0.0	0.0
1/100x	5	9	23.5	27.8	3.42	2.37	10.0	30.9
1/10x	6	20	26.5	14.3	3.66	1.65	3.7	52.0
1x	15	81	21.8	4.8	3.67	0.14	3.5	95.8
10 x	18	99	23.5	1.0	3.15	0.05	17.1	98.6
100x	39	99	14.8	1.3	1.64	0.11	56.8	96.7

Table 4. R11-AMACH and S-AMACH visual control, height, biomass, and biomass reduction 3 WAT with various rates of thifensulfuron (SU).

	Visual Control		Height		Biomass		Biomass Reduction	
RATE	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH
	%		cm		g		%	
0	0	0	33.8	30.5	3.80	3.43	0.0	0.0
1/100x	4	22	36.0	25.0	4.21	1.76	-10.8	48.7
1/10x	9	47	32.5	11.0	4.45	0.85	-17.1	75.2
1x	22	98	23.0	2.0	3.48	0.07	8.4	98.0
10 x	38	99	15.8	0.3	2.25	0.04	40.8	98.8
100x	64	99	6.8	0.5	0.58	0.03	84.7	99.1

Table 5. R11-AMACH and S-AMACH visual control, height, biomass, and biomass reduction 3 WAT with various rates of imazethapyr (IMI).

	Visual Control		Height		Biomass		Biomass Reduction	
RATE	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH
	%		cm		g		%	
0	0	0	33.8	30.5	3.80	3.43	0.0	0.0
1/100x	2	12	32.8	25.3	4.11	1.98	-8.2	42.3
1/10x	9	58	28.0	10.3	3.00	0.63	21.1	81.6
1x	16	97	20.3	2.3	2.62	0.06	31.0	98.3
10 x	62	99	6.8	0.3	0.50	0.10	86.8	97.1
100x	95	95	3.3	2.3	0.15	0.06	96.1	98.3

Table 6. R11-AMACH and S-AMACH visual control, height, biomass, and biomass reduction 3 WAT with various rates of pyrithiobac (POB).

	Visual Control		Height		Biomass		Biomass Reduction	
RATE	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH
	%		cm		g		%	
0	0	0	33.8	30.5	3.80	3.43	0.0	0.0
1/100x	7	16	26.5	21.8	2.78	1.73	26.8	49.6
1/10x	21	59	22.0	8.8	2.42	0.37	36.3	89.2
1x	30	99	17.8	2.0	2.38	0.07	37.4	98.0
10 x	48	99	11.0	1.8	1.07	0.12	71.8	96.5
100x	97	99	2.5	0.5	0.07	0.04	98.2	98.8

Table 7. R11-AMACH and S-AMACH visual control, height, biomass, and biomass reduction 3 WAT with various rates of cloransulam-methyl (TP).

RATE	Visual Control		Height		Biomass		Biomass Reduction	
	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH	R11-AMACH	S-AMACH
	%		cm		g		%	
0	0	0	46.8	34.4	10.83	8.53	0.0	0.0
1/100x	0	97	47.8	0.7	10.78	0.02	0.5	99.8
1/10x	23	91	34.5	3.6	8.16	0.25	24.7	97.1
1x	16	96	37.6	1.3	7.49	0.08	30.8	99.1
10 x	39	99	32.0	0.0	6.44	0.00	40.5	100.0
100x	63	99	12.2	0.0	2.68	0.00	75.3	100.0

Figure 1. R11-AMACH plants 3 WAT with chlorimuron (SU) at various rates.

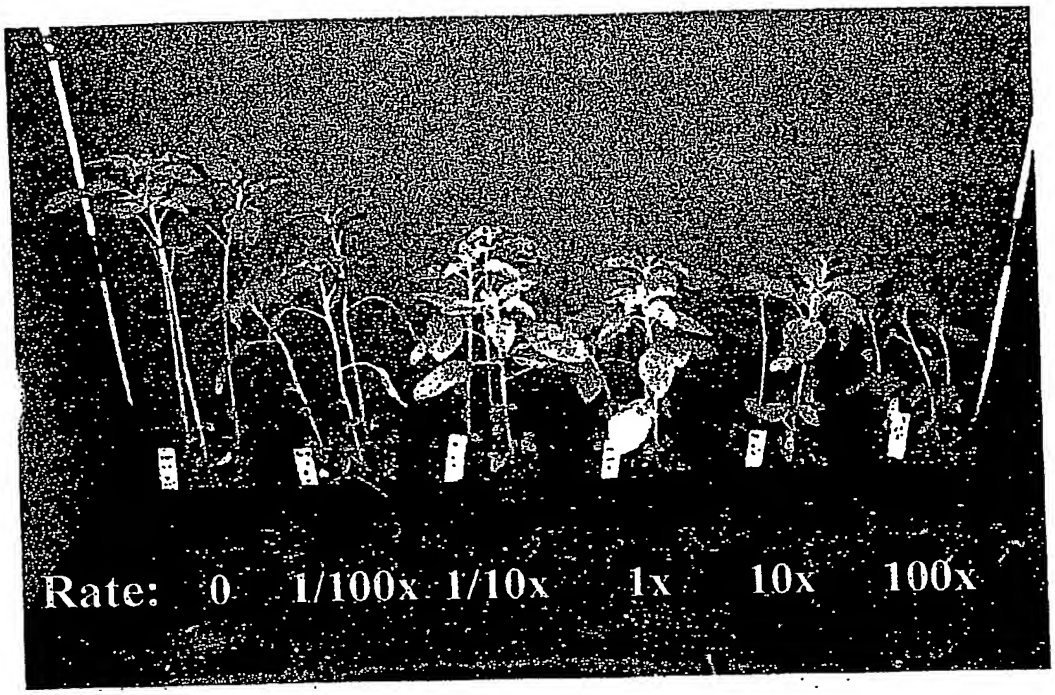

Figure 2. R11-AMACH plants 3 WAT with thifensulfuron (SU) at various rates.

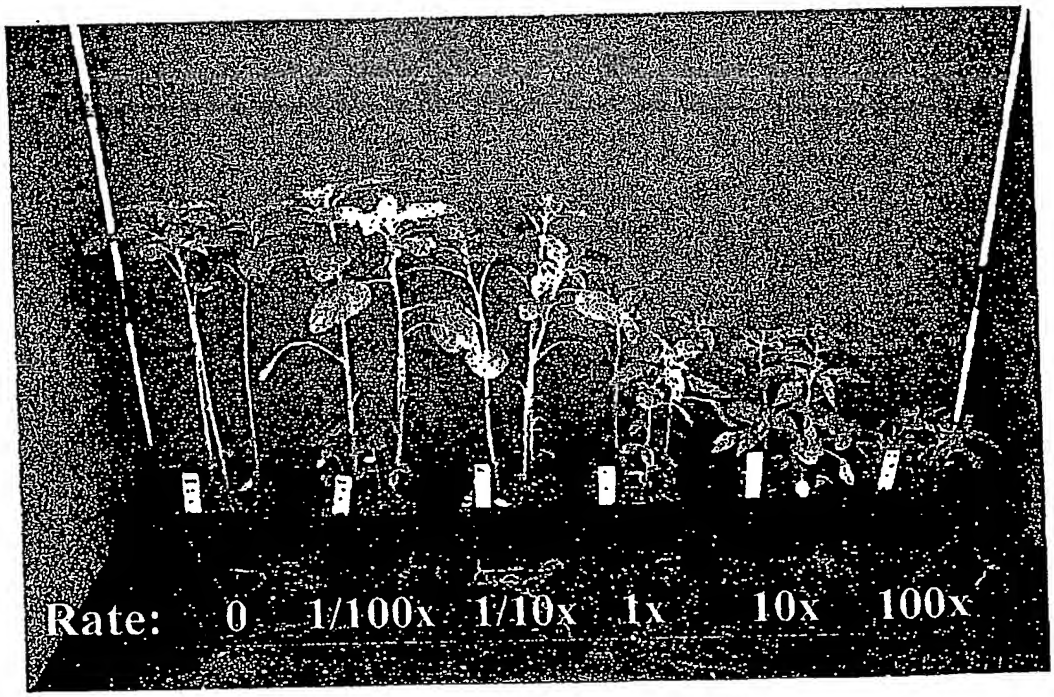

Figure 3. R11-AMACH plants 3 WAT with imazethapyr (IMI) at various rates.

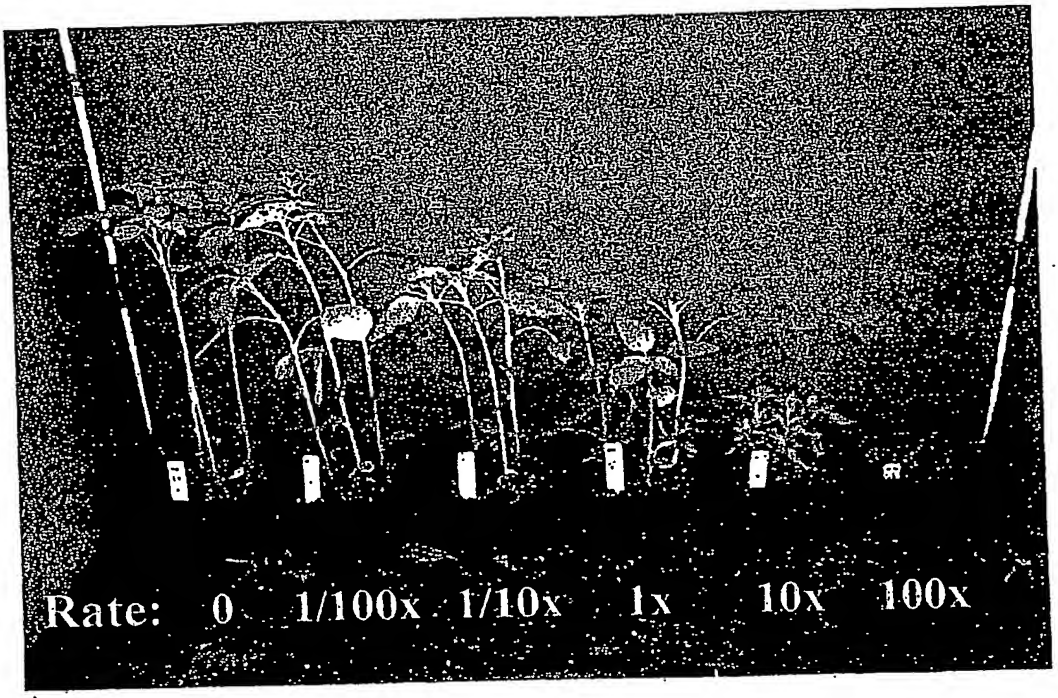

Figure 4. R11-AMACH plants 3 WAT with pyrithiobac (POB) at various rates.

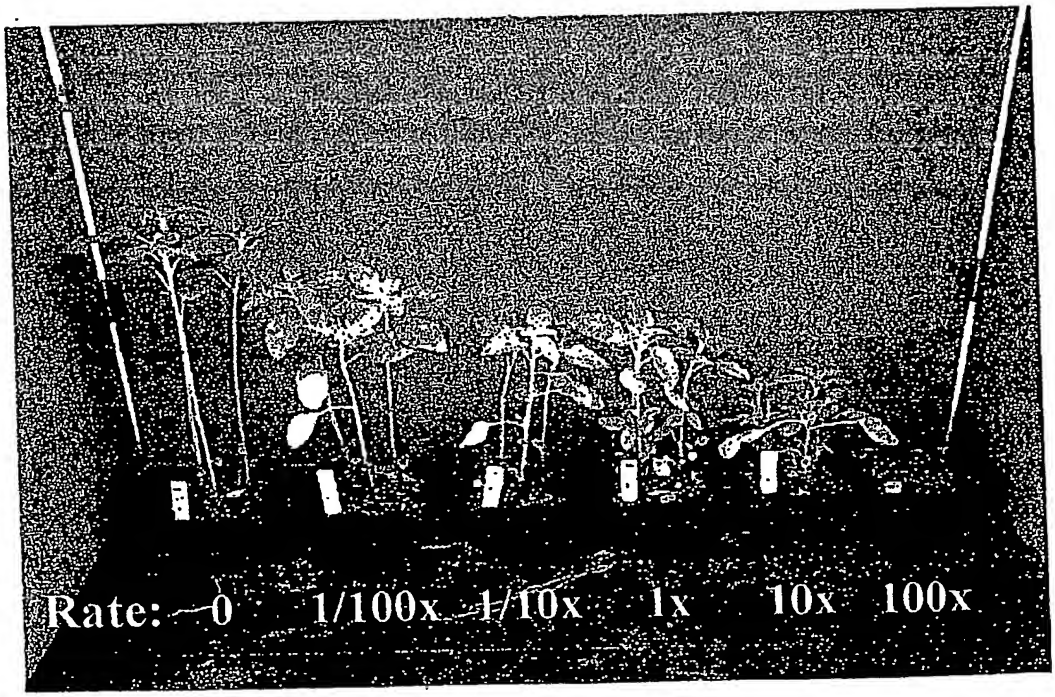

Figure 5. R11-AMACH plants 3 WAT with cloransulam (TP) at various rates.

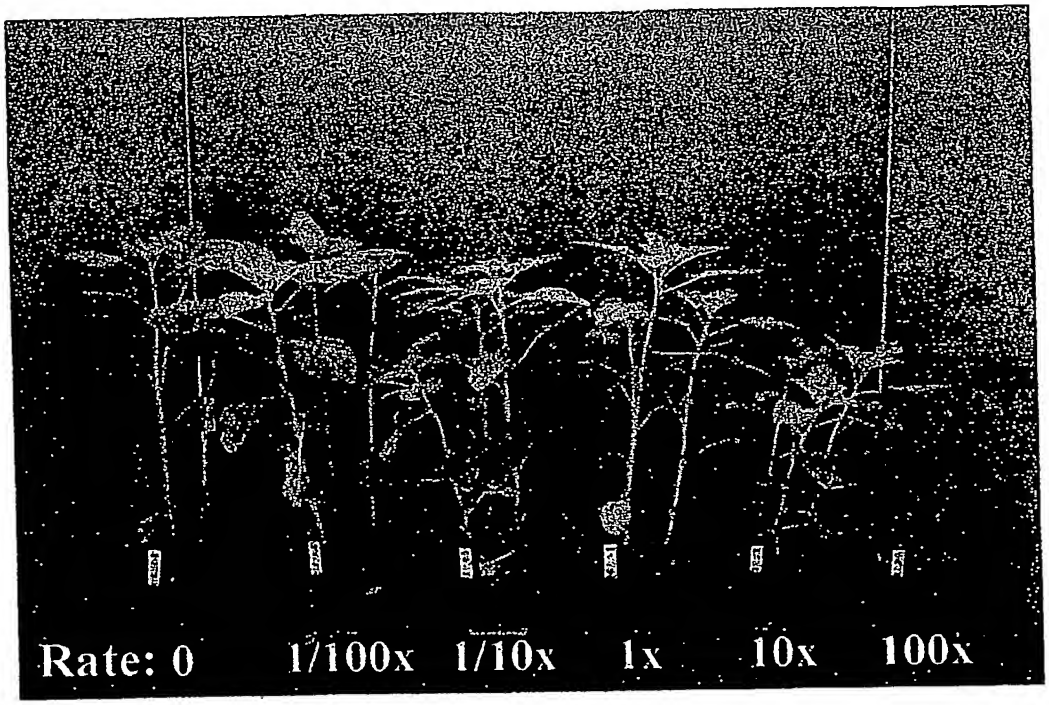

Figure 6a. R11-AMACH nucleotide sequence.

```
TCATCATCTTCTTCTCAATCACCTAAACCTAAACCTCCTTCCGCTACTATAACTCAATCACCTTCGTCTC 70
TCACCGATGATAAACCCTCTTCTTTTGTTTCCCGATTTAGCCCTGAAGAACCCAGAAAAGGTTGCGATGT 140
TCTCGTTGAAGCTCTTGAACGTGAAGGTGTTACCGATGTTTTTGCTTACCCTGGTGGAGCATCCATGGAA 210
ATTCATCAAGCTCTTACTCGTTCTAATATCATTAGAAATGTTCTTCCTCGACATGAACAAGGTGGGGTTT 280
TCGCTGCTGAAGGCTACGCTCGTGCTACTGGACGCGTTGGAGTTTGTATTGCCACTTCTGGTCCAGGTGC 350
TACTAATCTTGTTTCTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCCATTACTGGGCAA 420
GTTCCCCGGCGTATGATTGGTACTGATGCTTTTCAAGAGACTCCAATTGTTGAGGTAACTCGATCCATTA 490
CCAAGCATAATTATTTGGTGTTAGATGTTGAGGATATTCCTAGAATTGTTAAGGAAGCTTTCTTTTTAGC 560
TAATTCTGGTAGACCTGGACCTGTTTTGATTGATATTCCTAAAGATATTCAGCAACAATTAGTTGTTCCT 630
AATTGGGAACAGCCCATTAAATTGGGTGGGTATCTTTCTAGGTTGCCTAAACCCACTTATTCTGCTAATG 700
AAGAGGGACTTCTTGATCAAATTGTAAGGTTAGTGGGTGAGTCTAAGAGACCTGTGCTGTATACTGGAGG 770
TGGGTGTTTGAATTCTAGTGAAGAATTGAGGAAATTTGTCGAATTGACAGGTATTCCGGTGGCTAGTACT 840
TTAATGGGGTTGGGGGCTTTCCCTTGTACTGATGATTTATCTCTTCATATGTTGGGAATGCACGGGACTG 910
TGTACGCGAATTACGCGGTTGATAAGGCCGATTTGTTGCTTGCTTTTGGGGTTAGGTTTGATGAACGAGT 980
GACTGGTAAGCTCGAGGCGTTTGCTAGCCGGGCTAAGATTGTGCACATCGATATCGATTCTGCTGAAATC 1050
GGGAAGAATAAGCAACCTCATGTGTCGATTTGTGGTGATGTTAAAGTGGCATTACAGGGGTTGAATAAGA 1120
TTTTGGAATCTAGAAAAGGAAAGGTGAAATTGGATTTCTCTAATTGGAGGGAGGAGTTGAATGAGCAGAA 1190
AAAGAAGTTTCCTTTGAGTTTTAAGACTTTCGGGGATGCAATTCCTCCGCAATACGCCATTCAGGTTCTT 1260
GACGAGTTGACGAAGGGCGATGCGGTTGTAAGTACTGGTGTTGGGCAGCACCAAATGTGGGCTGCCCAAT 1330
TCTATAAGTACCGAAATCCTCGCCAATGGCTGACCTCGGGTGGTTTGGGGGCTATGGGGTTTGGTCTACC 1400
AGCTGCTATTGGAGCTGCTGTTGCTCGACCAGATGCGGTGGTTGTAGACATTGATGGGGATGGGAGTTTT 1470
ATCATGAATGTTCAAGAGTTGGCTACGATTAGGGTAGAGAATCTCCCGGTTAAAATCATGCTCTTGAACA 1540
ATCAACATTTAGGTATGGTTGTTCAATGGGAAGATCGATTTTACAAAGCTAACCGGGCACATACATACCT 1610
CGGGAATCCTTCCAATTCTTCCGAAATCTTCCCGGATATGCTCAAATTTGCTGAAGCATGTGATATACCA 1780
GCAGCCCGTGTTACCAAGGTGAGCGATTTAAGGGCTGCAATTCAAACAATGTTGGATACTCCAGGACCGT 1850
ATCTGCTGGATGTAATCGTACCACATCAGGAGCATGTGCTGCCTATGATCCCTAGCGGTGCCGCCTTCAA 1920
GGACACCATAACAGAGGGTGATGGAA                                              1966
```

Figure 6b. R11-AMACH residue protein.

```
SSSSQSPKPKPPSATITQSPSSLTDDKPSSFVSRFSPEEPRKGCDVLVEALEREGVTDVFAYPGGASMEIHQALTRS
NIIRNVLPRHEQGGVFAAEGYARATGRVGVCIATSGPGATNLVSGLADALLDSVPLVAITGQVPRRMIGTDAFQETP
IVEVTRSITKHNYLVLDVEDIPRIVKEAFFLANSGRPGPVLIDIPKDIQQQLVVPNWEQPIKLGGYLSRLPKPTYSA
NEEGLLDQIVRLVGESKRPVLYTGGGCLNSSEELRKFVELTGIPVASTLMGLGAFPCTDDLSLHMLGMHGTVYANYA
VDKADLLLAFGVRFDERVTGKLEAFASRAKIVHIDIDSAEIGKNKQPHVSICGDVKVALQGLNKILESRKGKVKLDF
SNWREELNEQKKKFPLSFKTFGDAIPPQYAIQVLDELTKGDAVVSTGVGQHQMWAAQFYKYRNPRQWLTSGGLGAMG
FGLPAAIGAAVARPDAVVVDIDGDGSFIMNVQELATIRVENLPVKIMLLNNQHLGMVVQWEDRFYKANRAHTYLGN
PSNSSEIFPDMLKFAEACDIPAARVTKVSDLRAAIQTMLDTPGPYLLDVIVPHQEHVLPMIPSGAAFKDTITEGDG
```

Figure 7a. S-AMACH nucleotide sequence.

```
TCATCATCTTCTTCTCAATCACCTAAACCTAAACCTCCTTCCGCTACTATAACTCAATCACCTTCGTCTCTC 70
ACCGATGATAAACCCTCTTCTTTTGTTTCCCGATTTAGCCCTGAAGAACCCAGAAAAGGTTGCGATGTTCTC 140
GTTGAAGCTCTTGAACGTGAAGGTGTTACCGATGTTTTTGCTTACCCTGGTGGAGCATCCATGGAAATTCAT 210
CAAGCTCTTACTCGTTCTAATATCATTAGAAATGTTCTTCCTCGACATGAACAAGGTGGGGTTTTCGCTGCT 280
GAAGGCTACGCTCGTGCTACTGGACGCGTTGGAGTTTGTATTGCCACTTCTGGTCCAGGTGCTACTAATCTT 350
GTTTCTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCCATTACTGGGCAAGTTCCCCGGCGT 490
ATGATTGGTACTGATGCTTTTCAAGAGACTCCAATTGTTGAGGTAACTCGATCCATTACCAAGCATAATTAT 560
TTGGTGTTAGATGTTGAGGATATTCCTAGAATTGTTAAGGAAGCTTTCTTTTTAGCTAATTCTGGTAGACCT 630
GGACCTGTTTTGATTGATATTCCTAAAGATATTCAGCAACAATTAGTTGTTCCTAATTGGGAACAGCCCATT 700
AAATTGGGTGGGTATCTTTCTAGGTTGCCTAAACCCACTTATTCTGCTAATGAAGAGGGACTTCTTGATCAA 770
ATTGTAAGGTTAGTGGGTGAGTCTAAGAGACCTGTGCTGTATACTGGAGGTGGGTGTTTGAATTCTAGTGAA 840
GAATTGAGGAAATTTGTCGAATTGACAGGTATTCCGGTGGCTAGTACTTTAATGGGGTTGGGGGCTTTCCCT 910
TGTACTGATGATTTATCTCTTCATATGTTGGGAATGCACGGGACTGTGTACGCGAATTACGCGGTTGATAAG 980
GCCGATTTGTTGCTTGCTTTTGGGGTTAGGTTTGATGATCGAGTGACTGGTAAGCTCGAGGCGTTTGCTAGC 1050
CGGGCTAAGATTGTGCACATCGATATCGATTCTGCTGAAATCGGGAAGAATAAGCAACCTCATGTGTCGATT 1120
TGTGGTGATGTTAAAGTGGCATTACAGGGGTTGAATAAGATTTTGGAATCTAGAAAAGGAAAGGTGAAATTG 1190
GATTTCTCTAATTGGAGGGAGGAGTTGAATGAGCAGAAAAAGAAGTTTCCTTTGAGTTTTAAGACTTTCGGG 1260
GATGCAATTCCTCCGCAATACGCCATTCAGGTTCTTGACGAGTTGACGAAGGGCGATGCGGTTGTAAGTACT 1330
GGTGTTGGGCAGCACCAAATGTGGGCTGCCCAATTCTATAAGTACCGAAATCCTCGCCAATGGCTGACCTCG 1400
GGTGGTTTGGGGGCTATGGGGTTTGGTCTACCAGCTGCTATTGGAGCTGCTGTTGCTCGACCAGATGCGGTG 1470
GTTGTAGACATTGATGGGGATGGGAGTTTTATCATGAATGTTCAAGAGTTGGCTACGATTAGGGTAGAGAAT 1540
CTCCCGGTTAAAATCATGCTCTTGAACAATCAACATTTAGGTATGGTTGTTCAATGGGAAGATCGATTTTAC 1610
AAAGCTAACCGGGCACATACATACCTCGGGAATCCTTCCAATTCTTCCGAAATCTTCCCGGATATGCTCAAA 1780
TTTGCTGAAGCATGTGATATACCAGCAGCCCGTGTTACCAAGGTGAGCGATTTAAGGGCTGCAATTCAAACA 1850
ATGTTGGATACTCCAGGACCGTATCTGCTGGATGTAATCGTACCACATCAGGAGCATGTGCTGCCTATGATC 1920
CCTAGCGGTGCCGCCTTCAAGGACACCATAACAGAGGGTGATGGAAGAAGGGCTTATTAGTTGGTTGGAGAT 1990
CTTTATAGAGGAGAAGCTTTTTTGTATGTATGTTAGTAGTTCCATAAACTTCTATATT 2046
```

Figure 7b. S-AMACH residue protein sequence.

```
SSSSSQSPKPKPPSATITQSPSSLTDDKPSSFVSRFSPEEPRKGCDVLVEALEREGVTDVFAYPGGASMEIHQALTR
SNIIRNVLPRHEQGGVFAAEGYARATGRVGVCIATSGPGATNLVSGLADALLDSVPLVAITGQVPRRMIGTDAFQET
PIVEVTRSITKHNYLVLDVEDIPRIVKEAFFLANSGRPGPVLIDIPKDIQQQLVVPNWEQPIKLGGYLSRLPKPTYS
ANEEGLLDQIVRLVGESKRPVLYTGGGCLNSSEELRKFVELTGIPVASTLMGLGAFPCTDDLSLHMLGMHGTVYANY
AVDKADLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGKNKQPHVSICGDVKVALQGLNKILESRKGKVKLD
FSNWREELNEQKKKFPLSFKTFGDAIPPQYAIQVLDELTKGDAVVSTGVGQHQMWAAQFYKYRNPRQWLTSGGLGAM
GFGLPAAIGAAVARPDAVVVDIDGDGSFIMNVQELATIRVENLPVKIMLLNNQHLGMVVQWEDRFYKANRAHTYLGN
PSNSSEIFPDMLKFAEACDIPAARVTKVSDLRAAIQTMLDTPGPYLLDVIVPHQEHVLPMIPSGAAFKDTITEGDGR
RAY
```

Figure 8. Amino acid sequence alignment of R11-AMACH and S-AMACH ALS gene. The mutation is indicated on top of the alignment (#) at position 375 within the highlighted region.

```
R11-AMACH    SSSSQSPKPKPPSATITQSPSSLTDDKPSSFVSRFSPEEPRKGCDVLVEA  101
S-AMACH      SSSSQSPKPKPPSATITQSPSSLTDDKPSSFVSRFSPEEPRKGCDVLVEA  101

R11-AMACH    LEREGVTDVFAYPGGASMEIHQALTRSNIIRNVLPRHEQGGVFAAEGYAR  151
S-AMACH      LEREGVTDVFAYPGGASMEIHQALTRSNIIRNVLPRHEQGGVFAAEGYAR  151

R11-AMACH    ATGRVGVCIATSGPGATNLVSGLADALLDSVPLVAITGQVPRRMIGTDAF  201
S-AMACH      ATGRVGVCIATSGPGATNLVSGLADALLDSVPLVAITGQVPRRMIGTDAF  201

R11-AMACH    QETPIVEVTRSITKHNYLVLDVEDIPRIVKEAFFLANSGRPGPVLIDIPK  251
S-AMACH      QETPIVEVTRSITKHNYLVLDVEDIPRIVKEAFFLANSGRPGPVLIDIPK  251

R11-AMACH    DIQQQLVVPNWEQPIKLGGYLSRLPKPTYSANEEGLLDQIVRLVGESKRP  301
S-AMACH      DIQQQLVVPNWEQPIKLGGYLSRLPKPTYSANEEGLLDQIVRLVGESKRP  301

R11-AMACH    VLYTGGGCLNSSEELRKFVELTGIPVASTLMGLGAFPCTDDLSLHMLGMH  351
S-AMACH      VLYTGGGCLNSSEELRKFVELTGIPVASTLMGLGAFPCTDDLSLHMLGMH  351
                                    #
R11-AMACH    GTVYANYAVDKADLLLAFGVRFDERVTGKLEAFASRAKIVHIDIDSAEIG  401
S-AMACH      GTVYANYAVDKADLLLAFGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIG  401

R11-AMACH    KNKQPHVSICGDVKVALQGLNKILESRKGKVKLDFSNWREELNEQKKKFP  451
S-AMACH      KNKQPHVSICGDVKVALQGLNKILESRKGKVKLDFSNWREELNEQKKKFP  451

R11-AMACH    LSFKTFGDAIPPQYAIQVLDELTKGDAVVSTGVGQHQMWAAQFYKYRNPR  501
S-AMACH      LSFKTFGDAIPPQYAIQVLDELTKGDAVVSTGVGQHQMWAAQFYKYRNPR  501

R11-AMACH    QWLTSGGLGAMGFGLPAAIGAAVARPDAVVVDIDGDGSFIMNVQELATIR  551
S-AMACH      QWLTSGGLGAMGFGLPAAIGAAVARPDAVVVDIDGDGSFIMNVQELATIR  551

R11-AMACH    VENLPVKIMLLNNQHLGMVVQWEDRFYKANRAHTYLGNPSNSSEIFPDML  601
S-AMACH      VENLPVKIMLLNNQHLGMVVQWEDRFYKANRAHTYLGNPSNSSEIFPDML  601

R11-AMACH    KFAEACDIPAARVTKVSDLRAAIQTMLDTPGPYLLDVIVPHQEHVLPMIP  651
S-AMACH      KFAEACDIPAARVTKVSDLRAAIQTMLDTPGPYLLDVIVPHQEHVLPMIP  651

R11-AMACH    SGAAFKDTITEGDGRRAY                                  669
S-AMACH      SGAAFKDTITEGDGRRAY                                  669
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Application papers not suitable for publication

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